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Sensitivity of cultivation of *Salmonella enterica* in pooled samples of pig faeces

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Summary: We aimed investigate if the cost of bacteriological examination at herd level could be reduced by cutting down on the number of analyses without loss of sensitivity. Faeces samples sent to the lab for bacteriological examination were analysed by both the standard procedure (20 pen samples) and by mixing the same 20 samples into 10, 5 or 1 pooled sample(s), respectively. The relative sensitivity of the bacteriological analysis decreased to 94% following pooling from 20 into 10; to 92% by pooling from 20 into 5; and to 73% following pooling from 20 into 1. Percent agreement between the standard procedure and pooled samples was >90% if only *Salmonella*-negative samples, or only *Salmonella*-positive samples, were pooled. Agreement was >60% for pools of originally negative and positive samples. The suggested alternative pooling methods should be carried out in the laboratory.

Keywords: bacteriological analysis, pooling, sensitivity, pig faeces

Introduction: In the Danish *Salmonella* surveillance programme, the prevalence of *Salmonella enterica* in finisher herds is monitored serologically at slaughter, by use of a meat juice ELISA. Follow up in herds with a moderate or high number of seropositive pigs, as well as their suppliers, consists of mandatory bacteriological examinations of 20 pen samples. A pen sample is defined as a 25 g sample, consisting of 5 x 5 g faeces collected from the pen floor. Samples are analysed in the laboratory for presence of *S. enterica*. The aim of the present study was to investigate whether the cost of bacteriological examination at herd level could be reduced by cutting down on the number of analyses without loss of sensitivity.

Materials and Methods: The study was carried out in the laboratory between 1 May and 30 September 2002. Routine samplings collected as described above and sent to the lab for bacteriological examination were analysed by both the standard procedure (20 pen samples) and by mixing the same 20 samples into 10, 5 or 1 pooled sample(s), respectively. All bacteriological examinations were qualitative, carried out according to standard procedures, including non-selective pre-enrichment and selective enrichment. Serotyping of *Salmonella* isolates was only carried out for the original 20 pen samples.

Results: During the study period, 51 pig herds that submitted 20 pen samples to the laboratory were found *Salmonella*-positive (at least 1 positive sample) and were included in the study. First, all 51 herds were re-examined by pooling from 20 pen samples into 10. Subsequently, 25 herds were also examined by pooling from 20 pen samples into 5, whereas the remaining 26 herds were examined by pooling from 20 pen samples into 1. The results of the different pooling procedures are shown in Table 1. It can be seen that the relative sensitivity of the bacteriological analysis decreases moderately following pooling from 20 into 10 or from 20 into 5; and quite remarkably following pooling from 20 into 1. Due to the poor sensitivity observed, the results of pooling 20 samples into 1 were not analysed any further.

Table 1. *Relative sensitivity of bacteriological analysis following examination of pooled faecal samples mixed according to different procedures.*

Procedure	Number of herds diagnosed Salmonella- positive*	Relative sensitivity (%)
20 pooled into 10	48 / 51	94.1
20 pooled into 5	23 / 25	92.0
20 pooled into 1	19 / 26	73.1

* Compared to herds diagnosed Salmonella-positive by the standard procedure

Serotypes found in the examination of 20 pen samples included Typhimurium, Derby, Infantis, Worthington, Ohio, Yoruba and Livingstone.

On an individual sample basis, pooling of only Salmonella-negative samples, or only Salmonella-positive samples, gave good agreement between the standard procedure and pooled samples (Table 2). When the pooled sample was a mixture of negative and positive samples (in different ratios) from the original 20 pen samples, the percent agreement dropped to 61-66% (Table 2).

Table 2. *Percent agreement of bacteriological results after pooling of all negative, mixed negative and positive, or all positive samples from the standard procedure.*

Combination of individual pen samples from standard procedure	Agreement (%) with standard procedure	
	20 pooled into 10	20 pooled into 5
All negative	95.4	95.5
Mixed (negative and positive)	66.2*	60.9*
All positive	91.6	100.0

* Salmonella-positive result

Discussion and conclusion: Our results indicate that pooling 20 pen samples to either 10 or 5 samples in the laboratory will not seriously compromise the sensitivity of the bacteriological examination of *Salmonella*-suspect swineherds. It seems likely, as it is with the present standard procedure, that herds both with low and high *Salmonella* levels are detected using this approach. Herds with very low *Salmonella* levels may go undetected, but this is also true for the current procedure including analysis of 20 pen samples. Pooling 20 samples into a single sample led to a significant reduction in sensitivity, which is not acceptable with regard to food safety.

At individual sample level the agreement between the *Salmonella* result after pooling and the *Salmonella* result in the original samples was high when only *Salmonella*-negative samples or only *Salmonella*-positive samples were mixed. When positive samples were mixed with negative samples, agreement was still >60% for both pooling 20 samples into 10 and into 5. This suggests that the quantitative amount of *Salmonella* present in the original positive samples was sufficient to also give a positive result after pooling in more than 60% of cases.

The suggested alternative pooling methods should be carried out in the laboratory. Veterinarians should still collect 20 pen samples in the herd using the standard procedure, to ensure that a representative number of pigs are sampled from each herd.

O 02 Surveillance of zoonotic bacteria in finishing pigs in The Netherlands

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Summary: In The Netherlands, from 1998 till 2002, a surveillance programme for zoonotic bacteria in finishing pigs was conducted at herd level. In 2000-2002, the prevalence of *Salmonella* spp. approximated 30%, while a significantly decreasing trend was observed when standardizing data for herdsize, age and quarter of sampling. Serotype discrimination showed the predominance of *S. Typhimurium* with an increasing role for phage type DT104. Prevalence estimates for *Campylobacter* spp. were 97% in 1998 (4th quarter only) and 45% in 1999. For STEC O157, prevalence estimates were 2% and 0% in 1998 and 1999, respectively. By using the samples from this study, a comparison study was conducted in which three different selective enrichment media, i.e. RV, MSRV and DIASALM, were compared for the isolation of *Salmonella* spp. from pig feces. Both MSRV and DIASALM scored significantly better compared to RV. By using logistic regression analysis of farm and herd specific data, potential risk factors for *Salmonella* spp. in finishing pig herds were identified and quantified.

Introduction: *Salmonella* spp. and *Campylobacter* spp. are recognized world-wide as important zoonotic bacteria causing gastro-enteritis in humans. In 1999-2000, a study was conducted in the Dutch general population indicating a total number of cases of campylobacteriosis and salmonellosis of approximately 100,000 and 50,000, respectively (de Wit et al., 2001). Based on typing results, it has been estimated that about one quarter of the human infections with *Salmonella* spp. in The Netherlands results from the consumption of pork or pork products (Van Pelt et al., 1999). In order to provide critical information for the control of zoonotic bacteria in primary production, a national programme for surveillance of *Salmonella* spp., *Campylobacter* spp. and Shiga-toxin-producing *Escherichia coli* (STEC) O157 in farm animals was implemented in 1997 (Bouwknegt et al., 2003). The main objectives of this programme are to monitor trends in the occurrence of these zoonotic micro-organisms and to identify risk factors for infection of farm animals. Finishing pigs were included in the programme from October 1998.

Materials and Methods: A two-stage sampling scheme was used for estimation of prevalence at herd level. Each year, the primary sample size (number of herds to be sampled) was calculated for estimation of the *Salmonella* prevalence based on the expected prevalence, a desired accuracy of 5% and a confidence level of 90%. Pig farms were randomly selected from a national database stratified according to farm size and geographical region. On each farm, one herd (i.e. finishing pigs housed in the same barn, usually divided in several compartments) was randomly selected for sampling. Yearly, approximately 150 to 200 herds of finishing pigs were sampled. However, due to an